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REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER U001AB	2. GOVT ACCESSION NO.	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) Advanced Biosensors for Amino Acid Detection		5. TYPE OF REPORT & PERIOD COVERED November 1-December 31, 1989
7. AUTHOR(s) R. L. Cook, R. C. MacDuff and A. F. Sammells		6. PERFORMING ORG. REPORT NUMBER N00014-89-C-0250
9. PERFORMING ORGANIZATION NAME AND ADDRESS Eltron Research, Inc. 4260 Westbrook Drive Aurora, IL 60504		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS
11. CONTROLLING OFFICE NAME AND ADDRESS Dr. Robert J. Nowak, Code 1113ES, Electrochemical Sciences Division, Office of Naval Research 800 N. Quincy St., Arlington, VA 22217-5000		12. REPORT DATE December 31, 1989
14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office) Above		13. NUMBER OF PAGES 16
		16. SECURITY CLASS. (of this report) Unclassified
		17a. DECLASSIFICATION/DOWNGRADING SCHEDULE
16. DISTRIBUTION STATEMENT (of this Report) Approved for public release, distribution unlimited.		
17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report) Approved for public release, distribution unlimited.		
18. SUPPLEMENTARY NOTES		
19. KEY WORDS (Continue on reverse side if necessary and identify by block number) amino acids, biosensors, fiber optic sensors (L-1)		
20. ABSTRACT (Continue on reverse side if necessary and identify by block number) Reversible optical detection has been achieved for the amino acids phenylalanine and 3-hydroxytyrosine using chromagenic acyclic polyether host molecules immobilized on a fiber optic substrate. For the hosts investigated tryptophan was found inactive which permitted selective phenylalanine recognition in its presence. Detection was achieved by continuous monitoring of evanescent wave interaction at the fiber optic/chromagenic host interfacial region.		

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Report #0001AB on ONR-SBIR Phase I Contract #N00014-89-C-0250

entitled

ADVANCED BIOSENSORS FOR AMINO ACID DETECTION

for the report period November 1 - December 31, 1989

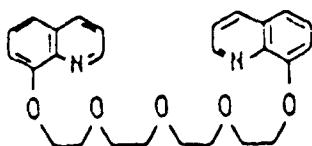
INTRODUCTION

The current research program is directed towards the development of optical waveguide biosensors (OWB) suitable for detecting both aromatic amino acids and biogenetic amines. The approach is based on the use of multifunctional host molecules which provide both a hydrophobic cavity suitable for initially binding the amino acid and a chromaphoric group whose optical properties will be perturbed by the resulting host/guest molecule interaction.

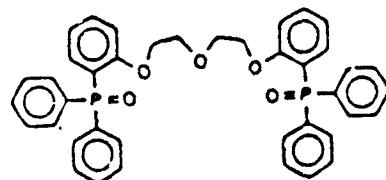
Desired characteristics of host molecules for facilitating the optical detection of amino acids include:

- The ability to conveniently reversibly bind the amino acid guest into the host molecule substrate.
- That the host/guest association will induce some detectable optical change.
- The host and host/guest molecular association are insoluble in aqueous electrolyte, with the amino acid guest being conveniently partitioned from the aqueous phase into a suitable semi-hydrophobic cavity.

The optical detection of amino acids during performance of this Phase I program has involved the selection as model pseudocavities of acyclic polyethers such as (I) or (II) as molecular hosts for reversible amino acid binding.



I



II

We were attracted to molecular hosts based upon I and II above because:

- the relative synthetic ease with which such molecular substrates may be prepared.¹
- the ability to tailor the host molecular substrate by varying either (or both) the polyether backbone or endgroups.
- the ability to incorporate into the polyether backbone a chromophore whose optical characteristics may be perturbed upon binding with an organic ammonium cation.²
- previously demonstrated and relatively rapid reversible binding kinetics afforded by these amine and amino acid hosts by the flexible polyether backbone.¹

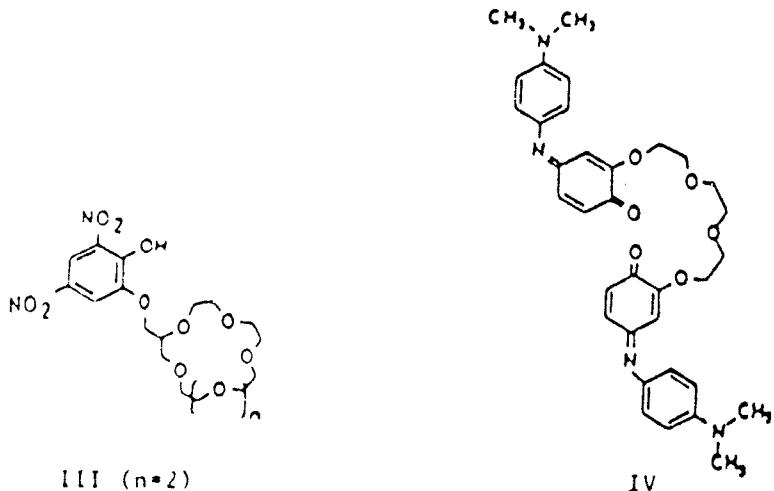
Selection of (I) as a molecular host for amino acids was prompted by previous work which has shown that inclusion of quinoline terminal groups onto acyclic polyether backbones can result in hosts possessing good stability for complexation with organic ammonium cations.³ Subsequent work has shown that the quinoline containing acyclic polyether (I) possesses high selectivity for binding, transport and release of amines and amino acids across an aqueous electrolyte/CHCl₃ liquid membrane/aqueous electrolyte cell⁴ when (I) is present

dissolved in the center chloroform layer. Here it was shown that transport of phentermine ($C_6H_5CH_2C(CH_3)_2NH_2$) or norephedrine ($C_6H_5CH(OH)CH(CH_3)NH_2$) by (I) occurs 170 and 62 times as rapidly respectively across a chloroform than either K^+ or Na^+ were transported.

The related molecular host (II) possessing phosphine oxide groups has high formation constants for hydrogen bond formation⁵ which, when incorporated into an appropriate molecular structure, would be expected to provide an anchoring site for organic ammonium cations. This has been previously demonstrated⁶ when (II) was dissolved in $CHCl_3$, where it was found effective for extracting phenylethylamine ($C_6H_5CH_2NH_2$) and dl-phenylglycine ethyl ester ($C_6H_6(NH_2)COOCH_2CH_3$) from 0.2N aqueous perchloric acid solutions. In this case, some extraction selectivity was observed in that neither methylamine, ethylamine, propylamine or n-butylamines were removed from the aqueous electrolyte.

In addition to molecular recognition and selectivity, a host substrate molecule incorporated as a sensitive surface in a chemical biosensor should ideally also exhibit reversibility to the analyte of interest. Acyclic polyethers in general have been shown to exhibit more rapid reversible kinetics for complexation than crown ethers.¹ This property has been attributed to the flexible nature of the acyclic polyether backbone. Thus as previously discussed, the ability of acyclic polyethers such as (I) to bind an amine, transport the amine across a $CHCl_3$ liquid membrane and then release the amine into an aqueous phase^{4,7} suggested to us that incorporation of a chromophore to the acyclic polyether backbone might form the basis of optically detecting bound amino acids.

Recent work has shown that chromophoric groups can be incorporated into molecular hosts to give ion selective colorimetric reagents.^{8a,8b} Here when a 1,2-dichloroethane solution of (III) was brought into contact with an aqueous solution containing Na^+ species, the organic phase turned from yellow ($\lambda_{max} = 273nm$) to violet ($\lambda_{max} = 430nm$).⁹ Similar wavelength shifts have also been reported for acyclic polyethers such as (IV).^{8a} In host substrates (III) and (IV), complexation with a metal cation has been shown to induce an electron density shift into or out of the chromophore, thus perturbing the chromophore wavelength maxima.



In the current research program, synthesis and evaluation of suitable chromagenic receptor sites has been directed towards the compounds (V) and (VI). The combination of a chromagenic receptor site whose spectral properties are modified upon binding of a suitable analyte with fiber optics technology provides a basis for the development of optical waveguide biosensors.

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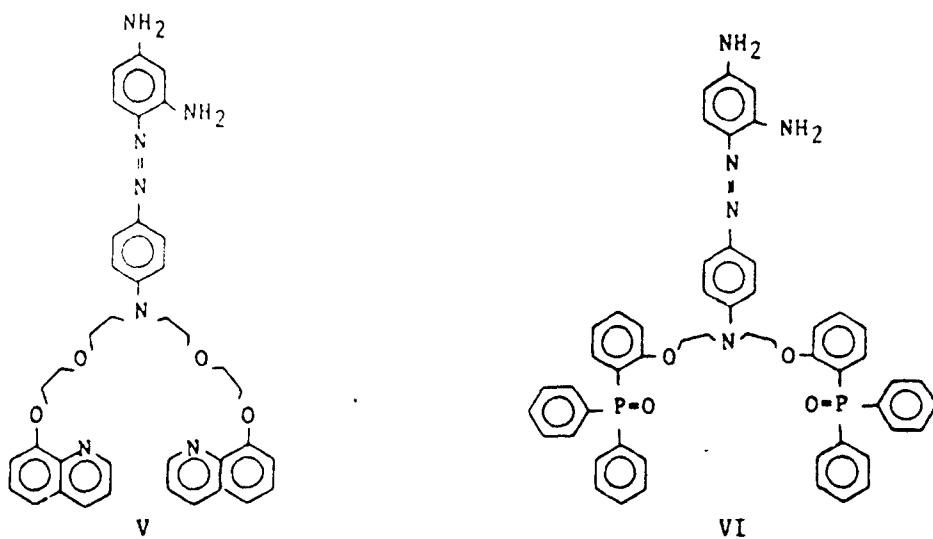


Figure 1 shows the basic construction of an optical fiber used in this program for amino acid detection. Fiber optic waveguides consist of an optically transparent inner core surrounded by an outer core having a lower refractive index than the inner core. A protective jacket encloses the

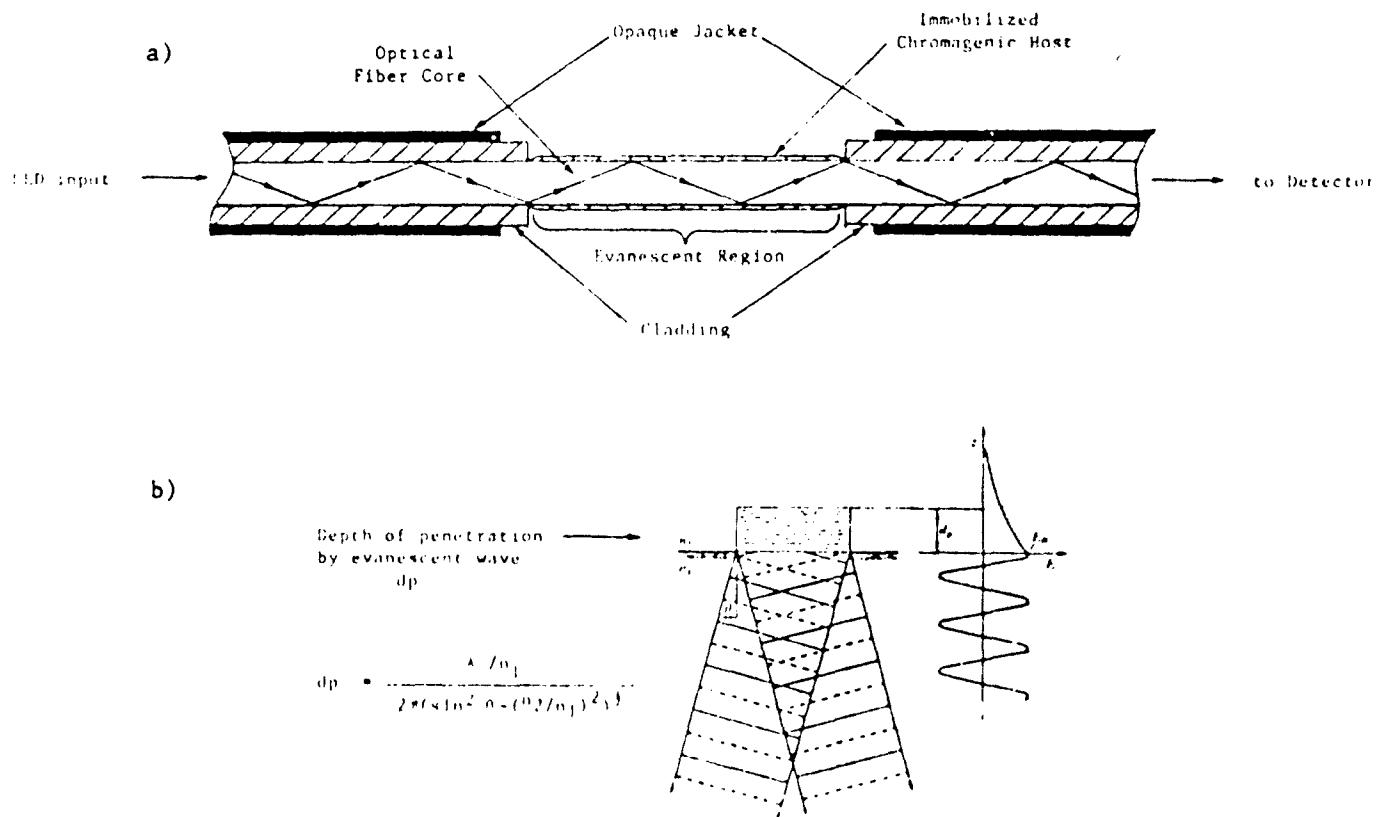


Figure 1. Schematic configuration for fiber optical detector (a) and generation of the evanescent wave at an interface between two optical media (b).

cladding and inner core. Light entering the fiber is totally internally reflected at the interface between the inner core and the cladding. Removal of cladding from the fiber optic permits some interaction with a medium external to the core. For a medium of lower refractive index interaction of optical energy can occur to a distance of $\sim 100\text{\AA}$. This corresponding light energy is known as the evanescent wave and optical sensors based on the use of this wave to probe the external environment are known as evanescent wave sensors. When the evanescent wave region is coated with a material whose interactions with the evanescent wave are modified by analyte binding, then a change in the light transmitted through the fiber is induced, permitting optical detection of analyte concentration.

During this reporting period we have performed preliminary work on an evanescent wave sensor based upon use of a plastic core optical fiber and coatings of the immobilized hosts (V) and (VI). The resulting optical sensor has been shown reversible towards detection of phenylalanine and dopa but not to tryptophan. The specific details of this work will now be discussed.

RESULTS

Task 1 Syntheses of Chromagenic Polyether Host Molecules

In this task the objective was to prepare compounds (V) and (VI) for incorporation onto a fiber optic for optical detection of dopa, phenylalanine and tryptophan. Discussion of the synthetic procedure for compound (V) is now presented.

Initial syntheses involved use of p-nitroaniline and 2-chloroethylether. Here 11ml (.112 moles) of 2-chloroethylether in 50ml DMF was added to 6.907g (0.05 moles) of p-nitroaniline also in DMF followed by refluxing overnight. However, subsequent gas chromatographic (GC) analysis of the reaction mixture using a Porapak Q column at 180°C showed essentially the same amount of 2-chloroethylether as in the starting mixture. Thus no reaction was promoted. Since resonance effects are important in aromatic amines and since p-nitroaniline is an extremely weak nucleophile, the lack of reactivity at this stage was probably due to low basicity of the amine function which made attack at the C-Cl bond unlikely.

This suggested that use of an electron donating group para to the amine might help promote the desired reaction. A commercially available amine of the desired structure was 4-aminoacetanilide. Here the acetamide group located para to the amino group was expected to donate electron density to the latter, thereby promoting the desired reaction. Furthermore, the acetyl part of the protecting group was available for convenient later removal via acid hydrolysis.

As an additional aid in promoting the desired reaction, we chose to add a base catalyst to the initial reaction mixture. Here ethyldiisopropylamine was chosen to promote the alkylation reaction at the nitrogen atom. Ethyldiisopropylamine is a strong sterically hindered base which would preclude its alkylation by 2-chloroethylether. Thus in the alkylation of amines, it could serve as a proton acceptor without becoming alkylated. Specific details of the synthetic scheme will now be discussed (Figure 2).

A) Condensation of 4-aminoacetanilide with 2-chloroethylether.

The first step involved addition of 7.50g of 4-aminoacetanilide in DMF to 11ml of 2-chloroethylether and 28ml of ethyldiisopropylamine during stirring.

The mixture was then heated to 100°C for 12 hours. The reaction mixture was then allowed to cool and DMF was removed under vacuum using a rotary evaporator to give a thick viscous liquid. This liquid was then dissolved into ~5ml of petroleum ether and separated on a neutral activated alumina column (Brockmann I, 150 mesh). A reddish-brown layer was collected and the petroleum evaporated to give 33% yield (6.69g) of (VII).

B) Addition of quinoline terminal end groups.

Compound (VII) (6.69g) in DMF was then added dropwise to a solution of 8-hydroxyquinoline (5.37g) in 30ml of DMF which in addition contained K_2CO_3 (5.1g). The mixture was then heated to 100°C overnight before cooling and filtering. DMF was then removed using a vacuum rotary evaporator. Reaction products were then separated on an alumina column as discussed above to give a reddish solid (compound VIII) in 41% yield (4.39g).

C) Removal of acetyl and introduction of chromagenic group.

Compound (VIII) (4.88g) was now dissolved in a 50/50 mixture of concentrated HCl/H_2O followed by heating to 50°C to remove the protecting acetyl group from the para-amino group. The solution was then cooled to 0°C using an ice bath. Cold aqueous sodium nitrite (0.7g) was now slowly added to the amine/ HCl mixture. To this mixture 1,2 phenylenediamine (1.8g) in 0.2N HCl was now added with vigorous stirring. Aqueous sodium acetate was added until compound (V), the desired chromagenic host, precipitated. The precipitate was then filtered, neutralized with 20-40% aqueous $NaOH$ and extracted into $CHCl_3$. Chloroform was evaporated and (V) purified using an alumina column and light petroleum ether as the eluant as discussed before. The yield for this final step was 52%, giving an overall yield of ~7% (2.6g). Elemental chemical analysis of the prepared compound agreed with that expected for the structure (V). (Galbraith)

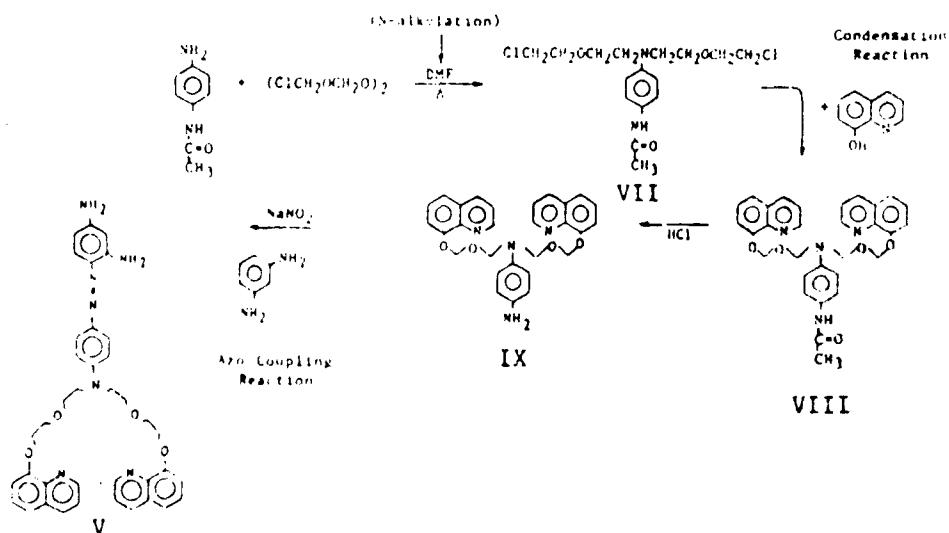


Figure 2. Summary of synthetic steps for preparation of quinoline based chromagenic host.

Synthesis of the phosphine oxide based chromagenic host (VI) will now be discussed (Figure 3). Magnesium (1.5g) was added to a THF solution (30ml) of 1-bromo-2-methoxybenzene (10g) and stirred until the Grignard reaction was initiated. After reaction, excess Mg was filtered and the solution cooled to -30°C. To this solution was added 11g of diphenylchlorophosphine in 20ml of THF. After stirring for 4 hours the reaction mixture was warmed to room temperature and stirred overnight before adding saturated potassium carbonate solution to the mixture. The liquid layers were separated using a separation funnel and the organic layer dried overnight using saturated CaSO_4 prior to filtration and removal of THF to give (o-methoxyphenyl) diphenylphosphine (X) in 75% yield (11.6g). The resulting (o-methoxyphenyl) diphenylphosphine was then oxidized with 30% H_2O_2 (5g) in acetone giving 11.0g of the phosphine oxide (XI). The methoxy group was now replaced with hydroxy by heating in chloroform with 5g of HI for 2-3 hours to give compound (XII).

The desired amino acid host site (VI) was prepared using the same method as for (V), except that 40g of bis(2-tosylethylether) is used instead of bis (2-chloroethylether). Here compound (XIII) was added to a THF solution of o-hydroxyphenyldiphenylphosphine oxide (XII) to which had been added an equimolar concentration of sodium ethoxide to catalyze the reaction. This mixture was then heated at 100°C for 6 hours, cooled and worked up as previously described for compound (VIII) to give (XIV) in 38% yield. Subsequent steps to give compound (VI) from (XIV) were carried out using procedures previously discussed in the synthesis of (V).

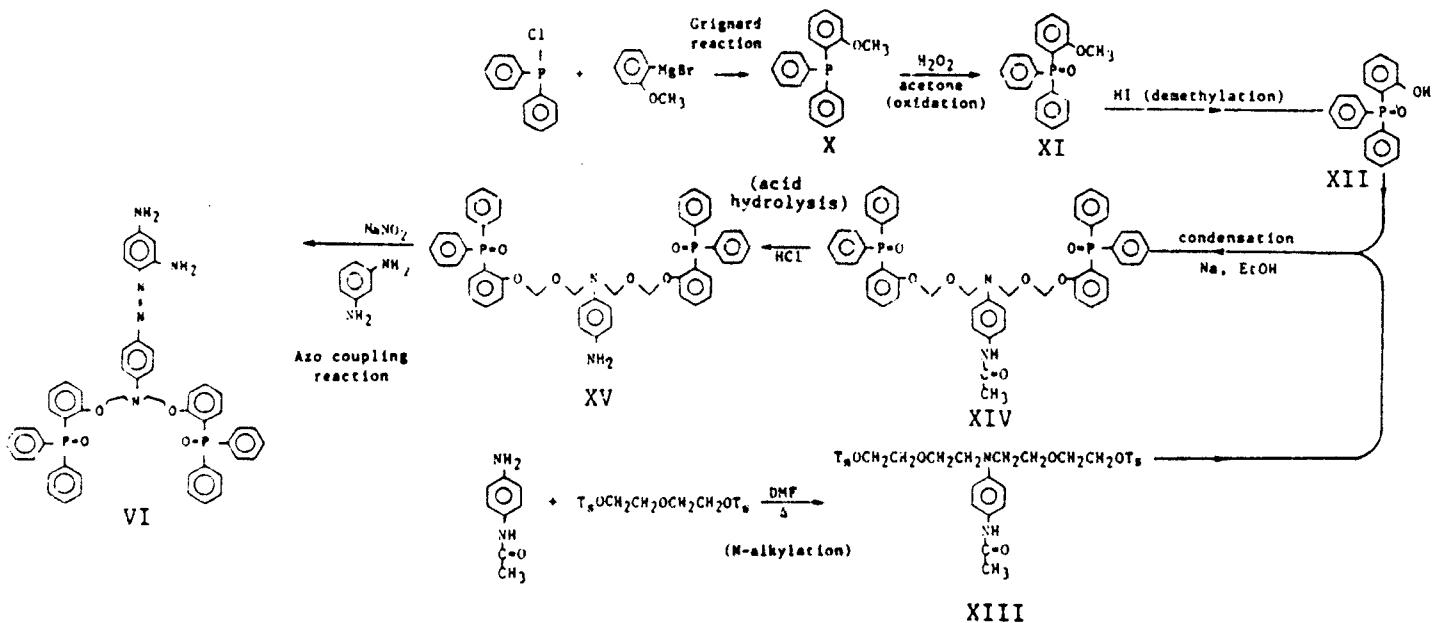


Figure 3. Summary of synthetic steps for preparation of phosphine oxide based chromagenic host.

Task 2 Determination of Wavelength Absorption Maxima for (V) and (VI) and Wavelength Shifts for V-Amine/Amino Acid and VI-Amine/Amino Acid Complexes

The objective of this task was to determine wavelength maxima for host compounds (V) and (VI) and corresponding wavelength shifts when exposed to

amino acid containing solutions. Here cellulose acetate (0.2g) was dissolved in 20ml of acetone along with 0.003g of the quinoline chromagenic host (V). This solution was then sprayed onto a glass slide using an airbrush and allowed to dry. The spray coated sides were then placed into a cuvette containing a solution of 0.1N HClO₄. A spectra was obtained using a Bausch and Lomb Spec-tronic 21 over the wavelengths of 350-950nm (solid line, Figure 4). This solution was then replaced with 0.1N KClO₄ containing 1g/200ml phenylalanine and allowed to set for 20 minutes prior to acquiring the spectra again over the wavelengths between 350-950nm (dotted line, Figure 4).

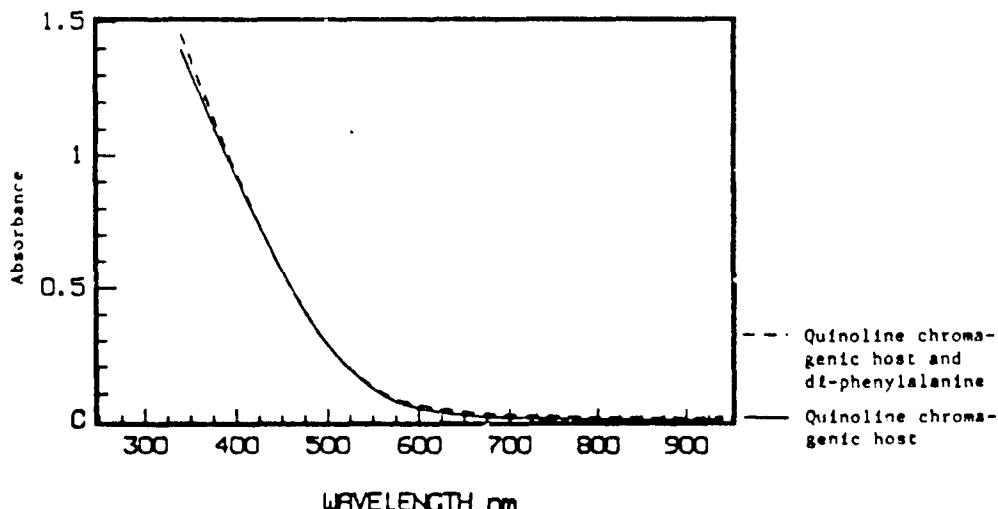


Figure 4. Absorbance vs wavelength plot for quinoline chromagenic host and quinoline chromagenic host plus phenylalanine.

As can be seen, no significant difference in the visible region of the spectra between host and host/guest complex was apparent. However, it may have been that changes may have occurred in the UV range. In any event, the depth of penetration by the evanescent wave (Figure 1) will be dependent in part upon relative refractive indices between the fiber optic (n_1) and that of the immobilized chromagenic host or host/guest complex (n_2). Hence perturbation in n_1/n_2 as a consequence of host/guest binding between the acyclic polyether and a given amino acid might be expected to be detectable by such changes in n_2 . Additionally inclusion of an amino acid guest into the chromagenic quinoline host's pseudocavity could be expected to result in modification of the dielectric properties of the host, thus also influencing the evanescent wave and leading to a signal change associated with amino acid binding. The possibility of such changes giving an observable signal were evaluated during performance of Task 3.

Task 3 Fabrication and Evaluation of (V) and (VI) in Cellulose Acetate Membranes Immobilized on Fiber Optic Probes for Amine and Amino Acid Analysis

The objective of this task was to determine the utility of immobilized chromagenic hosts (V) and (VI) present on the outside of an unclad optical fiber for the reversible optical detection of amino acids in aqueous solution. Amino acids of immediate interest in this Phase I effort included dl-phenylalanine, dl-dopa and dl-tryptophan.

We will now discuss electronics for fiber optic instrumentation, optic fiber coating procedures and detection results obtained for amino acid containing aqueous solutions.

A) Electronic circuitry for fiber optic sensor.

As seen from the schematic diagram (Figure 5), the transmitter circuit used is based around a 555 timer arranged in a basic astable circuit with an output of about 14kHz (2/3 on, 1/3 off). The output was connected to the power transistor D44HS used to drive the super bright LED. The LED, which has a peak emission at 660nm with a luminous intensity of 5000mcd, was coupled to a 1000 μ m fiber optic cable leading to the optical sensor and reference circuits.

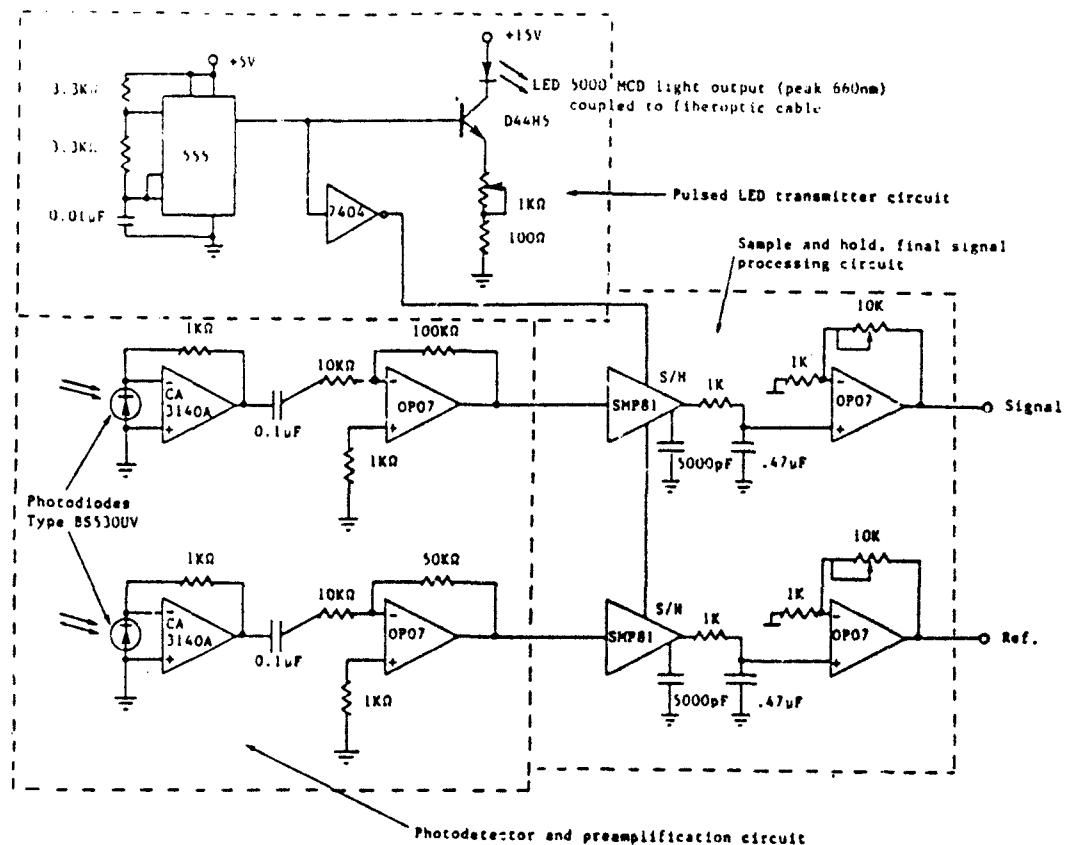


Figure 5. LED/Photodetector circuit for optical waveguide sensor.

The receiver was designed around two independent circuits, the first being the signal channel and the second the reference channel; both circuits were similar. Here light from sensor and reference cables were coupled into two Sharp BS530UV photodiodes. As seen from Figure 6, this detector had a very wide range of sensitivity, 200 \pm 1150nm with 500-900nm falling above 80% peak sensitivity. The use of signal and reference channels along with the use of sample and hold circuitry was chosen to provide low background drift and high signal to noise capability.

The photodiode was connected to the MOS/FET inputs of a CA3140A op. amp. The output was then fed through a DC blocking capacitor into an OP07 op. amp. circuit to further amplify the signal which was then fed into a SMP81 sample and hold (S/H) circuit. The S/H signal was derived from the timer chip 555, hence detection will be synchronous, permitting low levels of light changes to be detected. The sample and hold output is fed into a RC filter to remove

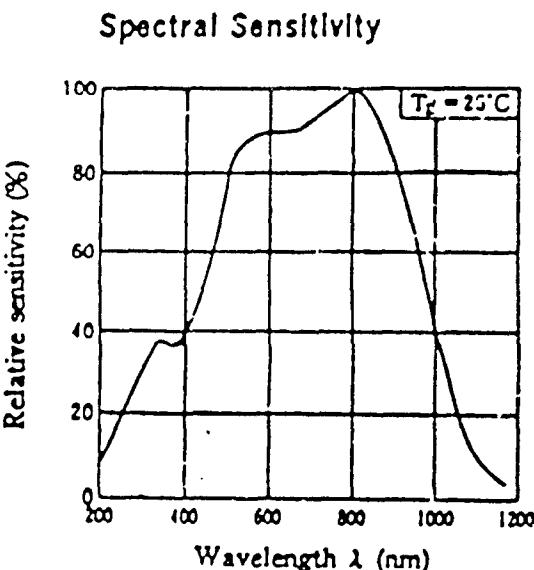


Figure 6. Spectral sensitivity of the B5530UV photodiode.

any transients. In the final stage the signal was amplified to give a voltage directly proportional to the light coming from the sensor.

The use of a reference signal channel compensated for the well known drift in LED output with temperature changes. As a consequence the signal channel was subtracted from the reference channel thereby producing a stable baseline signal.

In addition to the receiver/transmitter circuits, a digital data acquisition system (Figure 7) has been fabricated to acquire and process the sensor signal using software control. This data acquisition system consists of an analog to digital converter, memory and microprocessor. The microprocessor is an Intel 8052AH BASIC with built-in BASIC computer language, and an EPROM programmer, which is directly attached to a PC for data transfer.

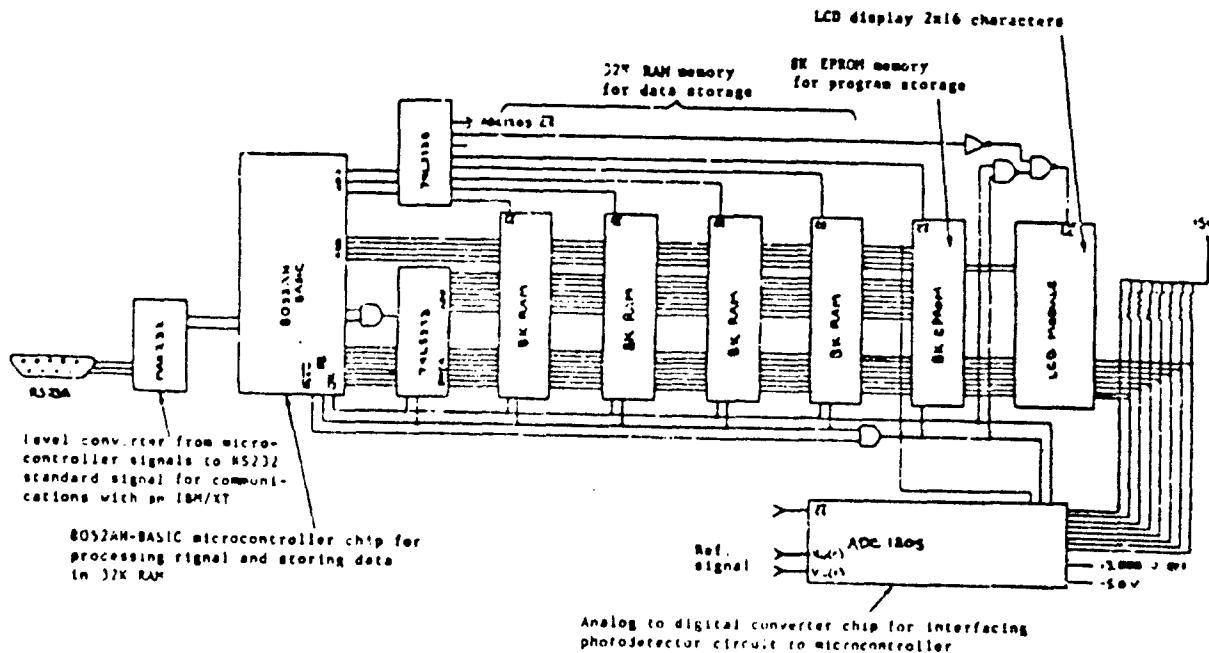


Figure 7. AD/DA converter circuitry for digital data acquisition.

Analog to digital conversion was performed using a National Semiconductor ADC1205CCJ chip which has a resolution of 12 bits plus sign and a conversion time of 100 μ s. Depending on the voltage window being sampled, resolution ranges from 1.2 to .12mV (Table 1) were achievable.

TABLE 1

Voltage window/resolution characteristics calculated at ERI for the national semiconductor ADC1205CCJ chip

Reference Voltage	Resolution
5.0V	1.2mV
4.5V	1.1mV
4.0V	1.0mV
3.5V	0.85mV
3.0V	0.73mV
2.5V	0.61mV
2.0V	0.49mV
1.5V	0.37mV
1.0V	0.24mV
.5V	0.12mV

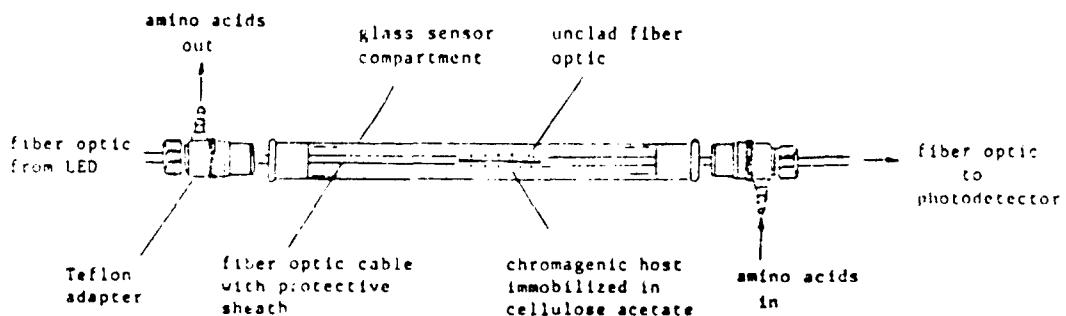
Finally, the microprocessor was wired to an alpha-numeric LCD module to monitor the photovoltage and other relevant information.

Initial operation of the prototype consists of zeroing the signal and reference channels while the optical sensor is "turned off". Once zeroed, the unknown amino acid guest molecule was introduced into the system via a peristaltic pump and the signal channel adjusted for signal optimization. This was then stored by the data acquisition system for subsequent graphic output.

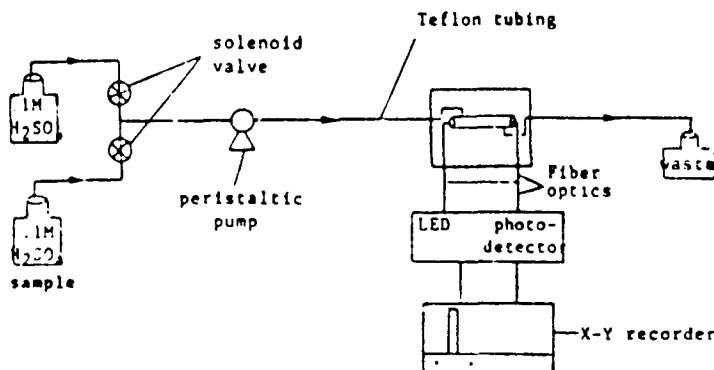
B) Preparation of fiber optic sensor.

The fiber optic cable used during this task has a 1000 μ m polymethylmethacrylate core. Generally a 2 inch section of the fiber optic cable jacket was initially removed carefully using a sharp blade and this section of the cable mounted into short, 3cm long, 7mm^o OD glass tubes (Figure 8a) to permit initial mounting in the sensor cell prior to incorporation in the experimental arrangement (Figure 8b). The exposed region of the fiber optic cable was lightly abraded with 220 grit fine aluminum oxide sandpaper to remove the cladding. This region was then sprayed with an acetone solution containing the chromagenic host and cellulose acetate.

In the experimental work performed to date the concentration of the chromagenic host was either 0.05 or 0.02g/20ml and the cellulose acetate 0.05g/20ml of solvent. The solvent used in all cases to date was acetone. The solution was spray-coated onto the fiber using an airbrush. A few passes were made along the length of the exposed fiber and the solvent allowed to evaporate prior to another deposit being made. This was repeated until ~10ml of the coating solution was used. The coated fiber was allowed to dry for 10-30 minutes and then mounted in the sensor cell compartment. The cell was then filled with electrolyte (0.1N HClO₄) using a peristaltic pump before the sensor circuitry was turned on. The solution was then passed through the cell at 12ml/min⁻¹ to provide a background signal. The flow was then switched to amino acid containing solutions while the voltage output from the photo-detector was monitored.



a) Fiber optic cell arrangement.



b) Overall experimental arrangement for introduction and removal of solution.

Figure 8. Experimental arrangement used for optical detection of amino acids.

c) Results of amino acid fiber optic sensor evaluation.

All measurements were performed with appropriate experimental controls. This included i) initially passing aqueous solution with and without the amino acid of interest past the exposed fiber optic, in the absence of any surface coating, ii) repeating the above experimental sequence with a sprayed cellulose acetate film but in the absence of an incorporated chromagenic host, and iii) again repeating the above experimental sequence with the chromagenic host incorporated within the cellulose acetate coating on the fiber optic surface. In all cases during this work no detectable optical response was found in the absence of a chromagenic host being incorporated within the cellulose acetate coating.

Figure 9 shows the results of the fiber optic coated with the chromagenic host (V) to 1g of phenylalanine in 200ml of 0.1N HClO₄. The fiber optic was initially coated with 0.005g of (V) dissolved within 20ml acetone. Upon passing the amino acid containing solution past the fiber optic, a distinct photovoltage absorption signal was observed corresponding to perturbation of the evanescence signal within the fiber optic. Upon replacing the amino acid containing HClO₄ with a solution containing only HClO₄ the optical signal was

removed. The observed response time was dictated by the solution flow rate into the sampling fiber optic cell. Most significant was the observation that these signals were 100% reversible.

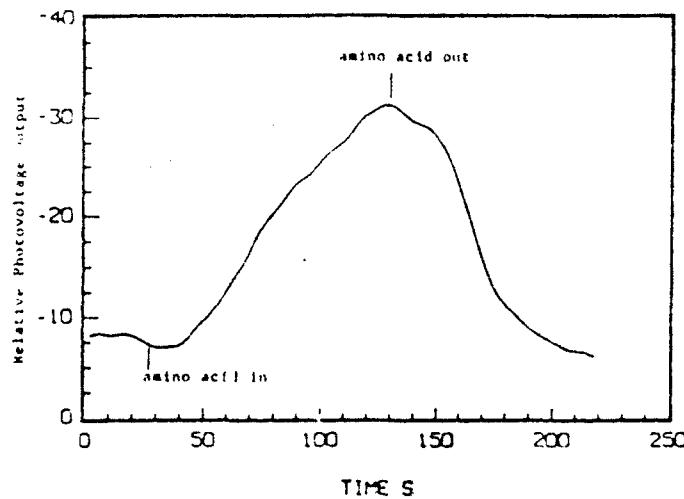


Figure 9. Phenylalanine response at evanescent wave optical sensor.
Solution = 1g phenylalanine in 200ml H₂O (0.1N HClO₄).
pH = 2-3, fiber was spray coated with 20ml of solution
containing .005g of quinoline chromagenic host, 0.05g
cellulose acetate. Flow rate of electrolyte 12ml/min.

As one might expect, incorporating a higher concentration of the chromagenic host within the cellulose acetate fiber on the fiber optic might result in signal enhancement. This expectation can be seen in Figure 10 where a four times higher concentration (0.02g) of the chromagenic host was used in the acetone/cellulose acetate spray solution.

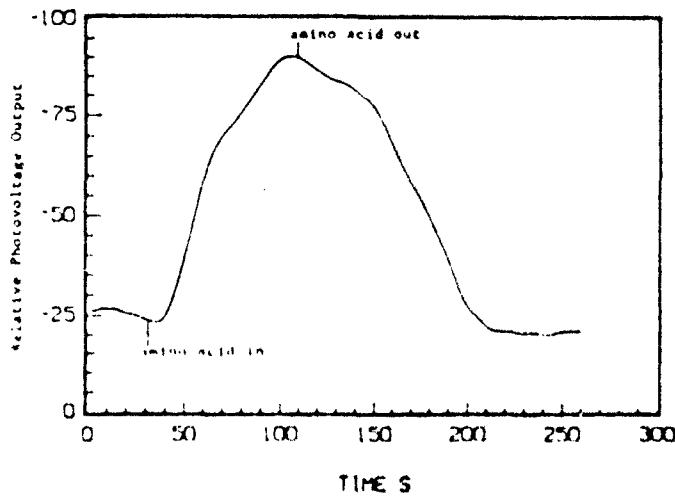
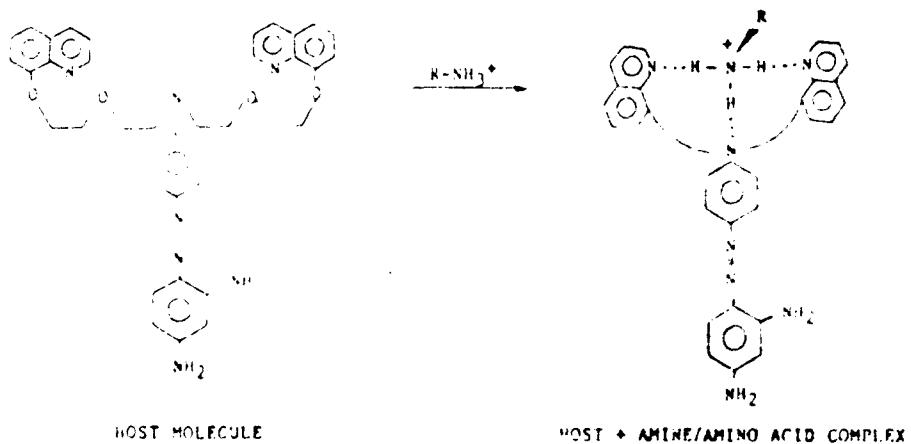


Figure 10. Phenylalanine response at evanescent wave optical sensor.
Solution = 1g phenylalanine in 200ml H₂O (0.1N HClO₄).
pH = 2-3, fiber was spray coated with 20ml of solution
containing .02g of quinoline chromagenic host, 0.05g
cellulose acetate. Flow rate of electrolyte 12ml/min.

This gives further evidence that the optical change observed is dependent upon the chromagenic host and may be explained by an association of the type:



Using the same coated fiber as in Figure 10 we tested the response to tryptophan. Interestingly no optical signal was detected. The experiment was now repeated using the following experimental sequence for passing aqueous solution through the fiber optic containing cell: 1) aqueous solution (0.1N HClO_4) containing no amino acid, 2) aqueous electrolyte containing tryptophan, and lastly 3) aqueous electrolyte containing both tryptophan and phenylalanine. The resulting response curves are summarized in Figure 11.

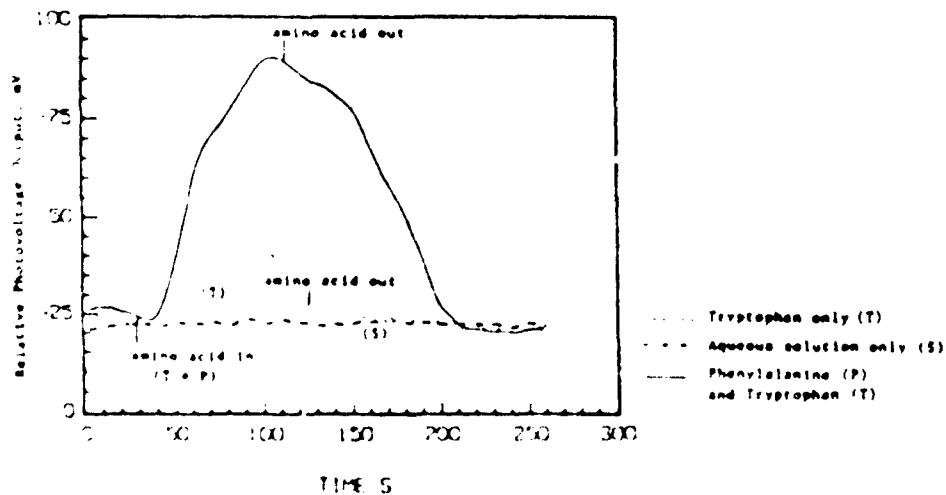
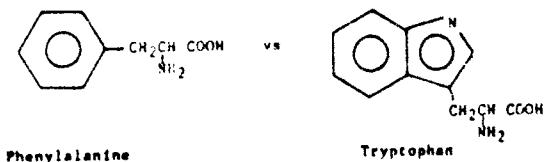


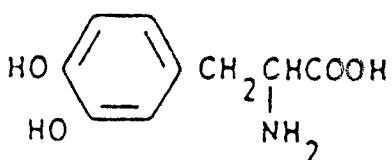
Figure 11. Comparison of optical waveguide sensor response to a) aqueous 0.1N HClO_4 , b) tryptophan (1g/200ml), and c) phenylalanine (1g/200ml) + tryptophan (1g/200ml) at an evanescent wave biosensor coated with quinoline chromagenic host. Solution flow rate 12ml/min.

The significant experimental observation here was that no optically detectable response was evident upon exposing the quinoline based host to tryptophan and that the corresponding signal for phenylalanine when using tryptophan/phenyl-

lanine mixtures was almost identical to that for phenylalanine solutions where tryptophan was absent (Figure 10). Thus, the chromagenic host (V) appears to show some selectivity towards dL-phenylalanine in the presence of tryptophan. The reason for this observation is not clear at this time, although one might invoke steric arguments as to why this may occur:



As has been previously discussed, the incorporation of hydroxyl groups onto the aromatic ring of a given amino acid would be expected to decrease cation hydrophobicity, thus resulting in a weakening of binding to the acyclic polyether host and a somewhat smaller expected optical signal. To investigate this further we have examined the optical response from the amino acid dL-dopa, also known as 3,4-dihydroxyphenylalanine or 3-hydroxytyrosine.



3-hydroxytyrosine (dopa)

The corresponding optical response curves for two loadings of chromagenic host (V) supported on the fiber optic are summarized in Figures 12 and 13. The actual loadings of chromagenic host deposited on different fiber optics was not known with great accuracy at this stage. Our general observation

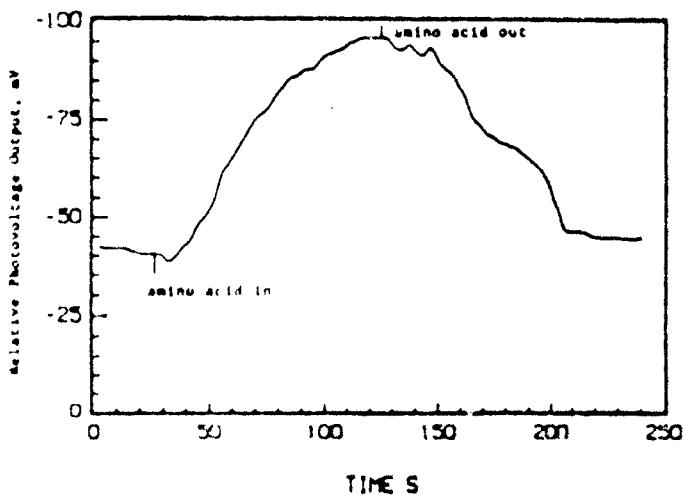


Figure 12. 3-hydroxytyrosine (dopa) response at an evanescent wave optical sensor. Solution = 1g/200ml H₂O (0.1N HClO₄). pH = 2-3. Fiber was spray coated with 20ml of solution containing .005g quinoline chromagenic host, .05g cellulose acetate. Electrolyte flow rate 12ml/min⁻¹.

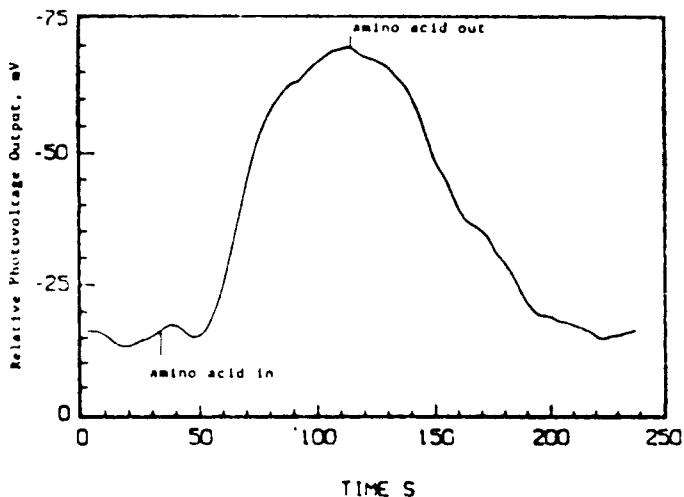


Figure 13. 3-hydroxytyrosine (dopa) response at an evanescent wave optical sensor. Solution = 1g/200ml H₂O (0.1N HClO₄). pH = 2-3. Fiber was spray coated with 20ml of solution containing .02g quinoline chromogenic host, .05 cellulose acetate. Electrolyte flow rate 10ml/min.

was that for fiber optics prepared in nominally the same manner, somewhat smaller optical signals were found for d₁-dopa compared to phenylalanine. In any event, the optical response obtained from the fiber optic supported chromogenic host when exposed to aqueous solution containing d₁-dopa was found to be totally reversible.

Finally we have just recently completed synthesis of the phosphine oxide chromogenic host (VI) and applied it to the fiber optic sensor for the detection of phenylalanine. Here although a good sensor response was observed, a significant baseline drift was present (Figure 14). It is not clear at this time whether this baseline drift was due to decomposition of the coating or associated with the electronics.

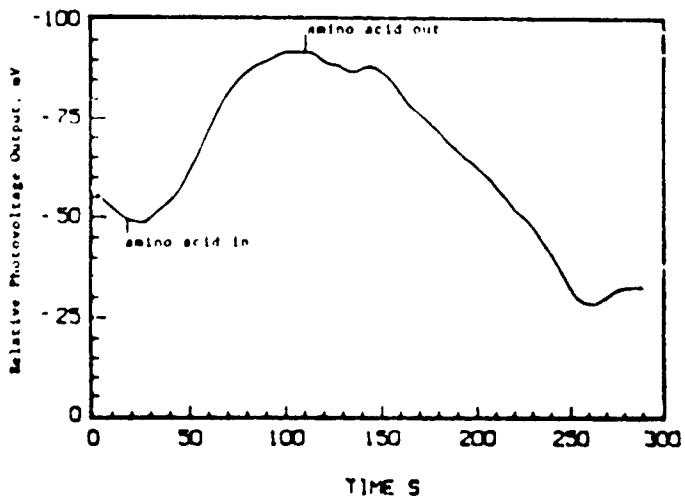
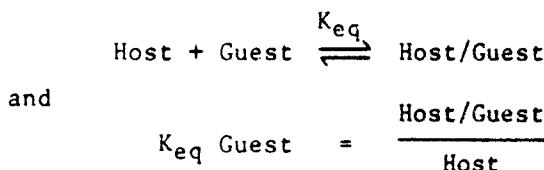


Figure 14. Phenylalanine response at an evanescent wave optical biosensor. Solution = 1g phenylalanine in 200ml H₂O (0.1N HClO₄). pH = 2-3. Fiber was spray coated with 20ml of solution containing .005g phosphine oxide chromogenic host and .05g cellulose acetate. Flow rate was 12ml/min.

The above discussed results are in support of chromagenic based host molecules supported on fiber optics as viable sites for achieving reversible detection of amino acids. In this report we have examined the optical sensor response for dl-phenylalanine, dl-dopa and dl-tryptophan at a concentration level of 1g/200ml (550ppm/mole). The response and sensitivity of the evanescent wave detector may be improved by i) increasing the length of the fiber optic/chromagenic host interaction region, and ii) increasing both the LED output and photodetector gain, detection levels of dl-phenylalanine and dl-dopa in the ppb/mole range (.001g/200ml) should be accessible using the current approach.

We have also begun to examine other options for the design of optical fiber sensor instrumentation. Here we can assume that the concentration of host and host/guest complex are related through the equilibrium:



Thus, if we can measure the concentration of host and host/guest simultaneously we can develop a fiber optic sensor whose output is a linear function of the guest concentration. Furthermore since K_{eq} will give a slope representing binding strength between the host and guest, then the application of appropriate software will be able to conveniently distinguish between guest molecules possessing various binding energies to the host. This goal may be addressed by utilizing two optical sources whose wavelengths are matched to the absorptions of the host and host/guest complex, respectively. Output from these sources will consequently be differentially absorbed by the host and host/guest complex, thus resulting in relative changes in the light exiting the fiber optic from which the guest concentration can be extrapolated.

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